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6. AUTHOR(S)
Russel H. Meints

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Oregon State University
Dept. of Botany & Plant Pathology
Corvallis, OR 97331-2902

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13. ABSTRACT *(Maximum 200 words)*

Eukaryotic marine algal viruses are large, dsDNA viruses. *Feldmannia* species Virus (resolved in two genome size classes 158 and 178 kbp) was developed as our prototype study systems. This virus infects marine brown algae. In nature sporophytic plants develop both plurilocular (mitotic) sporangia producing 2N spores and unilocular (meiotic) sporangia producing N spores. 2N spores normally yield adult sporophytes; haploid spores produce male and female gametophytes whose spores are the gametes for the sexual cycle. In the virus infected plant this life cycle is altered. Sporangia from virus-infected sporophytes do not produce spores. Instead unilocular sporangia contain virus particles. We show that the virus genomes exists in an integrated form within all other cells. All together the data suggest an integration/excision mechanism that employs an integrase/recombinase and conservative site-specific recombination. This enzyme complex appears to include topoisomerase-like activities which recognize sites within the virus and host. Unlike previously described systems we expect blunt end cutting and ligation or single bp. A large family of 173 bp repeat elements in the FsV genome was characterized. Two ORFs for "RING" zinc finger bearing genes were found as were two protein kinase genes. Northern blots demonstrated 6 major and 18 minor transcripts. The most abundant transcript was the major structural protein. Sequence analysis indicated significant homology with proteins of *Chlorella*-virus, Iridioviruses and African Swine Virus.

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FINAL REPORT

GRANT #: N00014-93-1-0251 Project No. 97-PR-0097-00

PRINCIPAL INVESTIGATOR: Dr. Russel H. Meints

INSTITUTION: Oregon State University

GRANT TITLE: Mechanisms of Viral Infection in Marine Brown Algae

REPORTING PERIOD: 1 January1996-31 December 1996

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OBJECTIVE: To carry out the first molecular characterization of a virus infecting a eukaryotic marine alga, and to determine whether this alga-virus system can be used as an experimental genetic tool for the study of the molecular genetics of brown algae (Phaeophyceae). To determine the insertion sites of the virus in the host genome and determine if this information could be exploited to develop a transformation system.

APPROACH: Virus-infected *Feldmannia* is maintained in laboratory culture. Viruses are purified from the cultures for study of virion structure and viral genome characteristics. The viral genome was cloned in a cosmid library to permit mapping of its structure, as source material and for probe analysis, and the nucleotide sequence of genes with abundant transcripts were determined to compare with known sequences and to provide promoter regions for construction of vectors for use in transformation experiments. Cosmid probing of gametophyte host DNA was provided the possibility of obtaining the virus insertion sites.

ACCOMPLISHMENTS (Final): Eukaryotic marine algal viruses are large, dsDNA viruses. *Feldmannia* species Virus (resolved in two genome size classes 158 and 178 kbp) was developed as our prototype study systems. This virus infects marine brown algae. In nature sporophytic plants develop both plurilocular (mitotic) sporangia producing 2N spores and unilocular (meiotic) sporangia producing N spores. 2N spores normally yield adult sporophytes; haploid spores produce male and female gametophytes whose spores are the gametes for the sexual cycle. In the virus infected plant this life cycle is altered. Sporangia from virus-infected sporophytes do not produce spores. Instead unilocular sporangia contain virus particles. We show that the virus genomes exists in an integrated form within all other cells. All together the data suggest an integration/excision mechanism that employs an integrase/recombinase and conservative site-specific recombination. This enzyme complex appears to include topoisomerase-like activities which recognize sites within the virus and host. Unlike previously described systems we expect blunt end cutting and ligation or single bp. A large family of 173 bp repeat elements in the FsV genome was characterized. Two ORFs for "RING" zinc finger bearing genes were found as were two protein kinase genes. Northern blots demonstrated 6 major and 18 minor transcripts. The most abundant transcript was the major structural protein. Sequence analysis indicated significant homology with proteins of *Chlorella*-virus, Iridioviruses and African Swine Virus.

SIGNIFICANCE: This first molecular characterization of a marine algal virus, and first investigation of the host range of a marine algal virus should provide important basic information about these poorly known marine pathogens. Development of a genetic transformation system for marine algae would greatly enhance the potential of these organisms for production of biomass energy and biopolymers through biotechnology.

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55. Lee, A. M., Ivey, R. G and Meints, R. H. Isolation and characterization of insertion sites (IS) from a dsDNA eukaryotic brown algal virus, FSV-1 from a marine brown alga, Feldmannia. (Manuscript complete)

56. Lee, A. M. and Meints, R. H. DNA polymerase gene characterization from FsV. (Manuscript complete)

57. Meints, R. H. (Studies underway) Genetics of normal and viral induced sporangial development in *Ectocarpus siliculosis*

R. H. Meints, PI
Oregon State University; 1997

The System

Filamentous brown alga

Viruses described (158 & 178 kbp)

Virus observed only in sporangium

Virus replication and expression

Objectives

Describe viral biology

Obtain viral insertion sites

Characterize viral fine structure

Develop mechanisms for transformation

Accomplishments

Completed map of two genome classes and subvarient of each

Completed isolation and characterization of insertion site

Completed transcript analysis and characterization of major structural protein, 2 "Ring" zinc finger proteins

Completed isolation and characterization of DNA polymerase gene, 2 protein kinases

Significance

First description of algal virus integration site

First description of marine algal virus major capsid protein

Progress toward development of transformation system